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# New Records of Phytophthora Diseases of Chinese Medicinal Herbs in Taiwan

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## ABSTRACT

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Three species of *Phytophthora* isolated from Chinese medicinal herbs grown in the fields during 2001-2009 were proven to be the new records of pathogens on these crops in Taiwan. They are *Phytophthora cryptogea* on dan-shen (*Salvia miltiorrhiza*), *P. drechsleri* on astragalus (huang-qi, *Astragalus membranaceus)* and *P. nicotianae* (=*P. parasitica*) on rehmannia (ti-huang, *Rehmannia glutinosa*). *Phytophthora cryptogea* attacked roots and basal stems of dan-shen, causing plant wilting, leaf drooping, and eventual plant death. *Phytophthora drechsleri* attacked leaves, stems and roots of astragalus, causing leaf and stem blight, and wilt and death of infected plants. Rehmannia was highly susceptible to *P. nicotianae*, as the infected plants were killed within a very short period of time. All the isolates of *P. cryptogea, P. drechsleri* and *P. nicotianae* obtained from the medicinal herbs in Taiwan belong to $A^1$ mating types. Results of the inoculation tests in the greenhouse showed that all the three species were pathogenic and the disease symptoms on inoculated host plants were similar to the naturally infected plants grown in the fields. Molecular data of the internal transcribed spacers (ITS) region and a partial sequence of the β-tubulin gene also support the traditional classification based on morphological and physical characteristics of *P. cryptogea, P. drechsleri* and *P. nicotianae. Phytophthora cryptogea* on dan-shen and *P. nicotianae* on rehmannia have not been reported in any other country prior to this report.

Key words：New disease, Chinese medicinal herbs, *Salvia miltiorrhiza*, *Astragalus membranaceus*, *Rehmannia glutinosa, Phytophthora cryptogea, P. drechsleri, P. nicotianae*, Taiwan.

# INTRODUCTION

Chinese medicinal herbs such as dan-shen (*Salvia miltiorrhiza*), astragalus (huang-qi, *Astragalus membranaceus*) and rehmannia (Ti-huang, *Rehmannia glutinosa*) are considered as important new emerging crops in Taiwan. Many famous species were continuously introduced from China and other countries. Diseases of these plants occurred subsequently and some of them caused substantial economic losses. However, only a few diseases of the Chinese medicinal herbs have been investigated in Taiwan [16]. Since the weather conditions in Taiwan are favorable for the occurrence of Phytophthora diseases [2], a survey of Phytophthora diseases on the three medicinal herbs, dan-shen, astragalus and rehmannia, was conducted to determine the pathogenicity and taxonomy of the *Phytophthora* pathogens on these plants in Taiwan. From the investigations, we reported the new records of Phytophthora diseases on the three species of medicinal herbs, including *Phytophthora cryptogea* Pethybridge & Lafferty on dan-sen, *P. drechsleri* Dastur on astragalus, and *P. nicotianae* Breda de Haan *(=P. parasitica* Dastur) on rehmannia. All the three Phytophthora diseases were first recorded in Taiwan.

# MATERIALS AND METHODS

## Isolation, maintenance and identification

Diseased plants of dan-sen, astragalus and rehmannia were collected from the fields of Taiwan from 2001 to 2009. Pieces of tissues were removed from diseased leaves (ca. 7 × 7 $mm^2$) and stems (ca. 5-10 mm long), disinfested with 0.5% NaClO for 30 sec and placed on the semi-selective medium, which consisted of 5% clarified V-8 juice agar (5% CV8A)(5% V-8 juice plus 0.2% $CaCO_3$ centrifuged at 1,500 rpm for 5 min, and 1.5% agar 【Hwei-shen Co., Changhua, Taiwan】) and supplemented with 200 ppm Ampicillin, 50 ppm mycostatin, and 10 ppm pentachloro-nitrobenzene (PCNB) after autoclave [13]. After incubation at 24℃ for 1-3 days, mycelial mats of *Phytophthora* species derived from the tissues were transferred onto 5% CV8A. Each fungus was purified by the single-zoospore culture technique. Single-zoospore cultures were used for morphological and molecular studies. The Classification Keys described by Waterhouse [19, 20] and the Tabular Key described by Stamp *et al.* [15] were used as the reference for morphological identification of the *Phytophthora* isolates obtained.

## Production of sporangia and zoospores

The method described by Hwang *et al.* [10] was used to produce large amount of sporangia for morphological studies and pathogenicity tests. Zoospore suspension of each tested *Phytophthora* isolate was prepared by the treatment at 15℃ for 30 min for chilling the mycelial mats with sporangia and then placing at 24℃ for 30 min for differentiation of sporangial cytoplasm and release of zoospores.

## Production of oospores and determination of mating types

Each isolate of *Phytophthora* was grown on 10% V8A (10% V-8 juice, 0.02% $CaCO_3$, 1.5% agar) at 24℃ in darkness for 10 days. Isolates capable of producing oospores in single cultures were designated as homothallic. Isolates which did not form oospores were each paired with the standard $A^1$ (p991) and $A^2$ (p731) mating type of *P. nicotianae* for determination of their mating types. Isolates producing oospores when paired with $A^2$ tester were designated as $A^1$ type; whereas isolates producing oospores when paired with $A^1$ tester were designated as $A^2$ type. For those isolates failing to form oospores while paired with either of the two testers, they were designated as neuter ($A^o$ type). Both isolates of p991 and p731 of *P. nicotianae* were courtesy of Dr. Zentmyer, the University of California, USA in 1980s.

The polycarbonate membrane method described by Ko[11] was used to study sexual reproduction of heterothallic species of *Phytophthora* and to determine their sexuality types [12].

## Growth of *Phytophthora*

To test growth rate of *Phytophthora* isolates, agar discs (5 mm diam.) containing mycelial mats were removed from the periphery of colonies of 3-5 day-old cultures grown on 5% V8A and inoculated on 5% CV8A in Petri dishes (1 disc/dish). The cultures were incubated at different temperatures (8, 10, 12, 16, 20, 24, 28, 32, 33, 35 or 36℃) in darkness and measured daily for mycelial growth until the colony reached the edge of the dishes or for a maximum of 10 days. The minimum, maximum and optimum growth temperatures for each tested isolates were determined. There were four dishes (replicates) for each temperature and the experiment was repeated once.

## Pathogenicity tests

**Preparation of inocula.** One or two isolates of *Phytophthora* obtained from each of the three hosts were selected for pathogenicity tests. Zoospore suspension (about $10^4$ zoospores/mL) was used as the inoculum.

**Preparation of inoculated plants.** Healthy seedlings (1-3 month old) of the three hosts were obtained from Crop Science Division, Taiwan Agricultural Research Institute, and grown in pots (9×9×12 $cm^3$) containing disinfested soils (1 seedling/pot).

**Inoculation.** For inoculation of each isolate of *Phytophthora* on leaves, 1-3 month old plants of astragalus and rehmannia were sprayed with zoospore suspension (10 mL/plant), and each plant was covered with a plastic bag for 2 days. Following removal of the plastic bags, the inoculated plants were kept in a growth room at 24℃ under light (12 hr)/dark (12 hr) and rated for disease incidence every 2 days until plants were killed or to a maximum of 14 days. Furthermore, diseased tissues were removed from the inoculated plants and used for reisolation of the pathogen. Two plants were inoculated for each *Phytophthora* isolate and the the experiment was repeated once. Plants treated with distilled water were used as controls.

For inoculation of each isolate of *Phytophthora* on stems, a piece of sterile cotton was wrapped around the stem of astragalus plants and inoculated by pipeting 0.5 mL of zoospore suspension onto the cotton. There were two plants for each *Phytophthora* isolate, with four inoculated stems per plant. The experiment was repeated once. Controls were treated with distilled water.

For inoculation of each isolate of *Phytophthora* on the basal stem of dan-shen, astragalus and rehmannia, the procedures were the same as those for inoculation of the stem. However, one mL of zoospore suspension was used as the inoculum and two plants were inoculated for each isolate. Plants treated with distilled water were used as controls.

## DNA sequencing of ITS1-5.8S-ITS2 region and partial β-tubulin region

**DNA preparation.** A piece of fresh mycelial block (ca. 3 mm × 3 mm × 2 mm) of the tested isolate of *Phytophthora* was placed at the center of a cellophane membrane, which was placed on top of 5%V8A in a Petri dish. After incubation at 24℃ for 7 days, mycelia on the surface of the cellophane membrane were harvested, lyophilized, and stored at -20℃ until use. The lyophilized mycelium (about 20 mg) was ground in liquid nitrogen and extracted for DNA using a Genomic DNA Purification Kit (GeneMark Technology Co., Taichung, Taiwan) according to the manufacturer's protocol.

**PCR amplification and DNA sequencing.** The DNA sequences of ribosomal internal transcribed spacer (ITS) regions (ITS1 and ITS2), including 5.8S coding region, plus partial 18S rRNA and 28S rRNA were amplified with the universal primers ITS5 (forward primer) and primer ITS4 (reversed primer) [21]. A partial sequence (658 bp) of the β-tubulin gene was also analyzed by amplification with the forward primer BT5 (5'-GTATCATGTGCACGTACTCGG) and the reversed primer BT6 (5'-CAAGAAAGCCTTACG ACGGA) developed by Villa *et al*. [18]. Direct sequencing of the PCR products was performed by the Seeing Bioscience Company (Taipei, Taiwan). For DNA sequencing of the ITS1-5.8SrDNA-ITS2 region, primers ITS5, ITS4 [21], 5.8S2-1 (5'-TCGCACATCGATGAAGAACG) and 5.8S2-2 (5'-TACGGACACTGATACAGGCAT) [5] were used, while

for sequencing of the partial sequence of the β-tubulin gene [18], primers BT5 and BT6 were used.

**Assembling of DNA sequences and GenBank searching.** The sequences of the ITS1-5.8SrDNA-ITS2 region and the partial sequence of the β-tubulin gene obtained from the original sequencing process were assembled and trimmed using the Vector NTI software V10.0 (InforMax Inc, CA, USA). The polymorphic portions were marked by IUPAC ambiguity codes. The query sequences were uploaded directly to the website of National Center of Biotechnology Information (NCBI: http://www.ncbi.nlm.nih.gov) and compared with sequences collected in the GenBank databases using the BLAST (Basic Local Alignment Search Tool) software to search for the closest *Phytophthora* species. Meanwhile, the sequences of ITS region and the partial sequences of β-tubulin gene of representative isolate of each *Phytophthora* species were submitted to GenBank.

# RESULTS

## Investigation and description of new Phytophthora diseases of medicinal herbs

Results of this study showed that the diseases on the Chinese medicinal herbs, dan-shen, astragalus and rehmannia, in the field survey during 2001-2009 were caused by *Phytophthora* species and they are newly recorded Phytophthora diseases on these plants. The Phytophthora diseases on dan-shen and astragalus were previously reported at the annual meeting of the Taiwan Phytopathological Society with an abstract published in Plant Pathology Bulletin by the authors [4]. All the Phytophthora diseases on these plants occurred in the fields during wet seasons. The disease was serious under continuous rainfall, resulting in serious economic losses. The hosts, pathogens and the numbers of isolates of *Phytophthora* collected from the Chinese medicinal herbs were summarized in Table 1.

### Phytophthora basal stem rot and root rot of *S. miltiorrhiza* caused by *P. cryptogea*

**Disease symptoms.** *Salvia miltiorrhiza* (dan-shen, 丹參**;** Lamiaceae 唇形科), an important species of medicinal herb grown in Mainland China (zh.wikipedia.org/zh-tw/丹參**)**, is regarded as an emerging herb in Taiwan. *Phytophthora* species was found to cause basal stem rot and root rot of dan-shen plants in several fields in Taiwan in the summer of 2004 due to continuous rainfall in that season. The diseased plants showed symptoms of leaf drooping in the early stage of infection and wilting of plants in 5-14 days (Fig. 1A). The internal tissues of infected basal stems turned dark brown initially and collapsed and rotted completely (Fig. 1B) at the late stage, resulting in plant death.

Table 1. New records of *Phytophthora* species isolated from diseased tissues of *Salvia miltiorrhiza*, *Astragalus membranaceus* and *Rehmannia glutinosa* in Taiwan

| Name of host (Scientific, English & Chinese) | Infected sites | *Phytophthora* species | Location | No. of isolates & mating type | Year of isolation | Disease severity[1] |
|---|---|---|---|---|---|---|
| *Salvia miltiorrhiza*, dan shen, 丹參 | Basal stem and root | *P. cryptogea* | Taichung | 2A[1] | 2004, 2005 | +++[1] |
| *Astragalus membranaceus*, 黃耆 | Leaf, stem and root | *P. drechsleri* | Taichung | 2 A[1] | 2006 | ++ |
| *Rehmannia glutinosa* 地黃 | Leaf, stem and root | *P. nicotianae* | Taichung | 10A[1] | 2008 | +++ |

[1] Disease severity observed in the field: +++, serious; ++: moderate; +: slight.

Fig. 1. Symptoms of Phytophthora diseases on medicinal herbs: dan-shen (*Salvia miltiorrhiza*) (A, B), astragalus (*Astragalus membranaceus*) (C, D) and rehmannia (*Rehmannia glutinosa*) (E, F). Note wilt (A) and root rot (B) of dan-shen caused by *Phytophthora cryptogea*; leaf blight (C) and stem rot (D) of astragalus caused by *Phytophthora drechsleri*; and basal stem rot (E) and root rot (F) of rehmannia caused by *Phytophthora nicotianae*.

**Characteristics of pathogen.** *Phytophthora cryptogea* was isolated from diseased dan-shen plants in two fields, one at Wufong, Taichung in 2004 and another at Yingge, New Taipei city in 2005. Both isolates of *P. cryptogea* were of the $A^1$ mating type and they belonged to Ko's sexuality type 2 [(12)], because the isolates induced the $A^2$ tester p731 of *P. nicotianae* to form oospores but failed to induce the formation of oospores by selfing. Mycelia of these *P. cryptogea* isolates grew slightly radiate on 5% CV8A, but formed rose-petal pattern on potato dextrose agar (PDA) in petri dishes (Fig. 2A). These isolates failed to produce sporangia on solid V8A in Petri dishes and only formed a few sporangia in distilled water. However, the mycelia in 5% V8 broth produced abundant sporangia after washing with mineral solution [(10)]. Sporangiophores were branched simple sympodially. Each sporangium bore 1-3 sporangia. Sporangia were ovate to obpyriform, non-papillate (Fig. 3A & B), and non-deciduous. Chlamydospores and sexual

organs were not observed. However, hyphal swellings resembling swollen irregular vesicles were observed on cultures in distilled water. (Fig. 3C)

The minimum and maximum temperatures for mycelial growth were 8℃and 32-33℃, respectively, with optimum temperature of 20-28℃ (Fig. 4). The morphological and physiological characteristics of isolate p24148 mostly fitted the description in the tabular key of Stamp's taxonomy [15] and the original description of specimen collected in Waterhouse' publication [20]. The data of the tested isolate were listed in Figs. 2, 3 and 4, and Table 2.

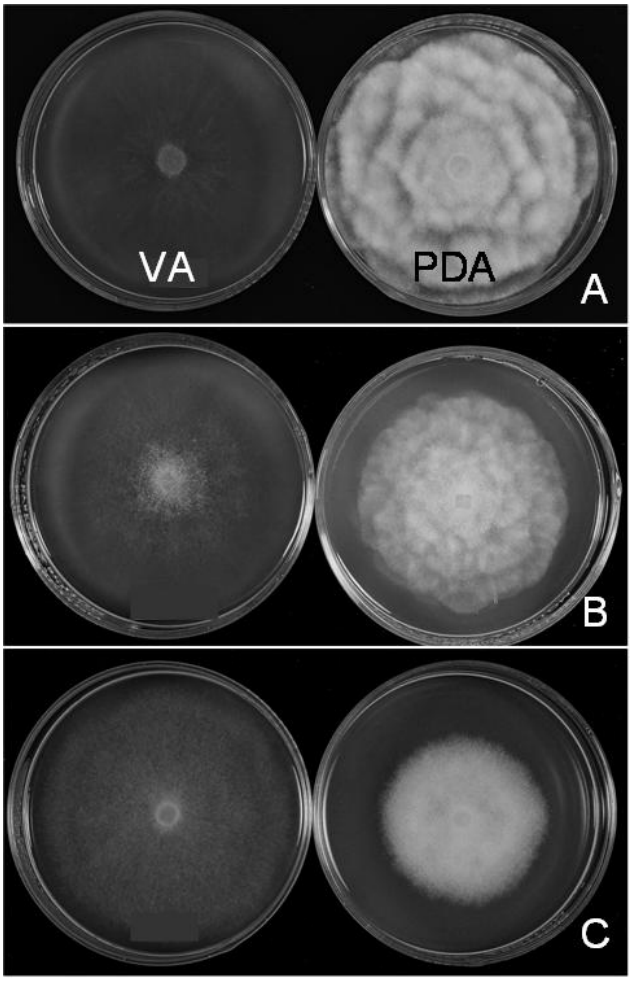


Fig. 2. Colony morphology of *Phytophthora cryptogea* isolate p24148 from *Salvia miltiorrhiza* (A), *P. drechsleri* isolate p26082 from *Astragalus membranaceus* (B), and *P. nicotianae* isolate p28065 from *Rehmannia glutinosa* (C). Cultures were grown on PDA (right plate) or 5% Clarified V-8 juice agar (left plate) at 24℃ for 5 days.

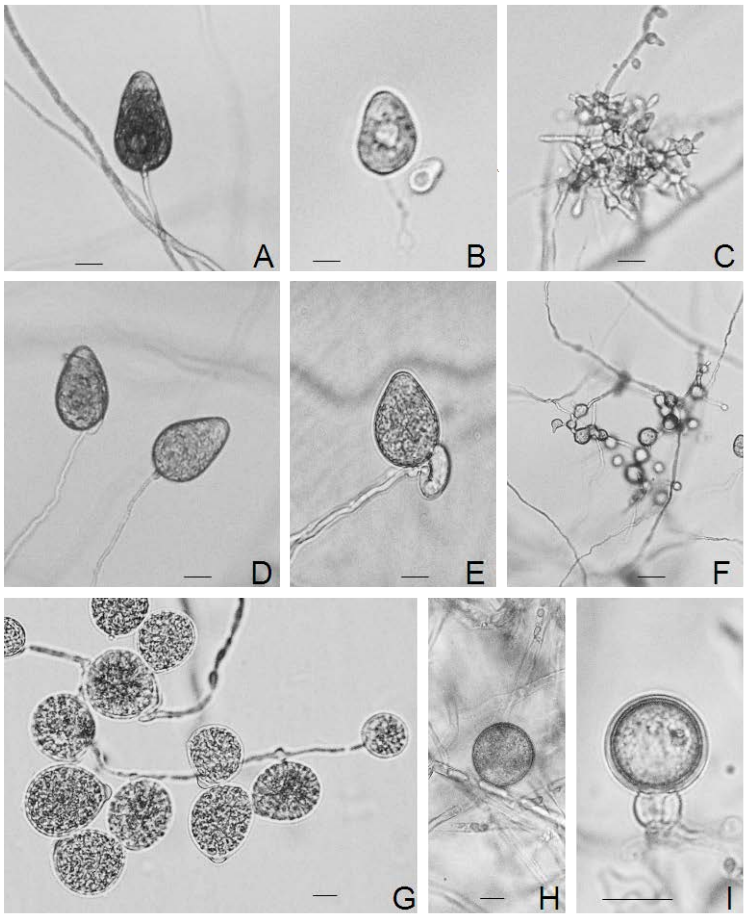


Fig. 3. Morphology of isolates of three species of *Phytophthora* from Chinese medicinal herbs. Note sporangia (A, B) and hyphal swellings (C) of *Phytophthora cryptogea* isolate p24148 from *Salvia miltiorrhiza;* sporangia (D, E) and hyphal swellings (F) of *P. drechsleri* isolate p26082 from *Astragalus membranaceus;* and sporangia (G), a chlamydospore (H) and an oospore (I) of *P. nicotianae* isolate p28065 from *Rehmannia glutinosa.* Bar = 20 μm.

**Pathogenicity.** Inoculation of the basal stem of 1-3 month old seedlings (5-10 leaves) of dan-shen with *P. cryptogea* resulted in the development of symptoms of leaf droopy and plant wilt similar to the naturally infected plants in the field in 7-14 days. The pathogen was reisolated from the stems of inoculated plants. The pathogen also caused leaf spots on the seedlings inoculated by spraying zoospore suspension on the whole plant.

**Leaf and stem blight, and root rot of** *A. membranaceus*, **caused by *P. drechsleri***

**Disease symptoms.** *Astragalus membranaceus*, (astragalus or huang gi, 黃耆, Leguminosae 豆科), a native species of perennial medicinal herb in northern China, has recently been grown in a small area in Taiwan. In 2006, an astragalus field at Wufong, Taichung, showed diseased plants with symptoms of severe leaf and stem blight (Fig. 1C & D). Greenish brown spots developed on small leaves initially and the infected leaves fell to the ground in 3-5 days. Many shoots and stems were also infected with brown lesions at the infected sites initially and the plants withered and died gradually. Infected root tissues were necrotic with brown discoloration and rotted.

**Characteristics of pathogen.** Two isolates of *P. drechsleri* were obtained from the diseased astragalus plants, one isolate from a stem and the other from a root. Both isolates of *P. drechsleri* were of the $A^1$ mating type and belonged to Ko's sexuality type 2 [12], because these isolates stimulated the $A^2$ tester to form oospores but failed to form oospores by selfing. Mycelia of these *P. drechsleri* isolates grew smoothly with a thin layer of sparse aerial mycelia on 5% CV8A, but formed rose-petal pattern on PDA in Petri dishes (Fig.2B). Similar to *P. cryptogea*, the isolates of *P. drechsleri* failed to produce sporangia on solid V8A in Petri dishes and only formed a few sporangia in distilled water. However, the mycelia in V8 broth produced abundant sporangia after washing with mineral solution [10]. The morphology of sporangia of *P. drechsleri* is slightly similar to *P. cryptogea*. Sporangiophores were branched simple sympodially. Sporangia were ovoid-shaped to obpyriform, symmetrical, non-papillate, and non-deciduous (Fig. 3D & E, Table 2). Sometimes, a thick tubular structure grew from sporangiophore near the sporangial base (Fig. 3E). Chlamydospores were not observed. Sexual organs were absent. Same as *P. cryptogea*, hyphal swellings resemble swollen irregular vesicles were observed on cultures of *P. drechsleri* grown in distilled water. (Fig. 3F)

The minimum and maximum temperatures for mycelial growth were 8 and 36℃, respectively, with an optimum temperature of 28-32℃ (Fig. 4). The morphological and physiological characteristics of *P. drechsleri* isolate p26082 mostly fitted the description in the tabular key of Stamp *et al.*' taxonomy [15] and the original description of specimen documented in Waterhouse' publication [20].

**Pathogenicity.** Spraying zoospore suspension of each isolate of *P. drechsleri* on 1-3 month old seedlings (5-10 cm high) of astragalus resulted in the development of leaf and stem blight symptoms in 3-7 days. Meanwhile, the pathogen also caused basal stem rot and root rot in 2-4 weeks, when the inoculum was applied to the basal stem of astragalus seedlings. The blight symptoms of leaf blight and root necrosis on inoculated seedlings were similar to those observed on naturally infected plants in the field. The pathogen was reisolated from all the infected tissues of the inoculated seedlings.

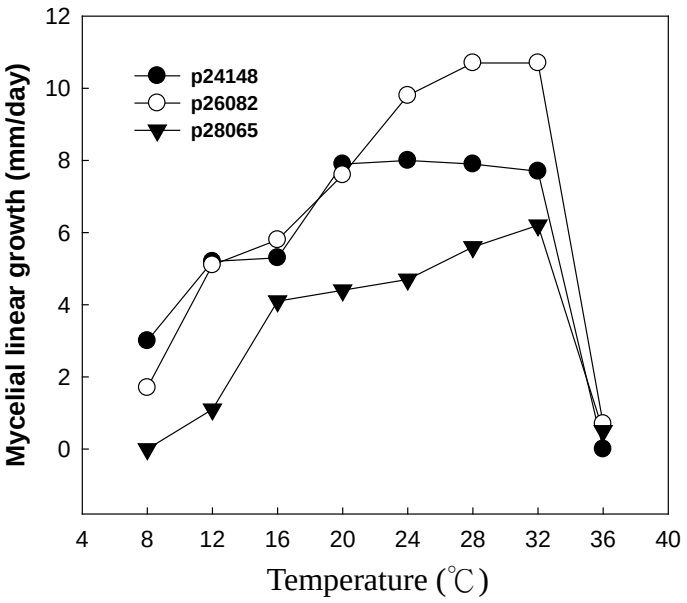


Fig. 4. Effect of temperature on linear growth curves of mycelia of *Phytophthora cryptogea* isolate p24148 from *Salvia miltiorrhiza*, *P. drechsleri* isolate p26082 from *Astragalus membranaceus*, and *P. nicotianae* isolate p28065 from *Rehmannia glutinosa*. Cultures were grown on 5% clarified V-8 juice agar in Petri dishes for 5 days.

Table 2. Size of sporangia and chlamydospores of *Phytophthora* isolates from *Salvia miltiorrhiza*, *Astragalus membranaceus* and *Rehmannia glutinosa*

| *Phytophthora* species | Isolate | Host | Sporangia | | Chlamydospores (μm) |
|---|---|---|---|---|---|
| | | | Length × Width (μm) | Length/width | |
| *P. cryptogea* | 24148 | *Salvia miltiorrhiza* | 45-(56.4)-65.4 × 30-(35.7)-40[1] | 1.25-(1.59)-2.17 | None[2] |
| *P. drechsleri* | 26082 | *Astragalus membranaceus* | 37.5-(42.4)-52.5 × 25-(31.3)-35 | 1.21-(1.36)-1.5 | None |
| *P. nicotianae* | 28065 | *Rehmannia glutinosa* | 40-(46.8)-60 × 32.5-(38.8)-45 | 1.13-(1.25)-1.57 | 29.5-(35.5)-45 |

[1] Data in parenthesis refer to means.

[2] No chlamydospores were observed.

## Phytophthora blight of *R. glutinosa* caused by *P. nicotianae*

**Symptoms.** *Rehmannia glutinosa* (rehmannia or ti-huang, 地黃; Orobanchacea 列當科) is a popular Chinese medicinal herb mainly grown in northern China (zh.wikipedia.org/zh-tw/地黃). A serious death of young plants of rehmannia was found at Wufong, Taichung in 2008 and 2009 (Fig. 1E & F). The infected plants showed symptoms of leaf drooping initially and the plants wilted and died in 3-7 days. The plants with severe leaf drooping also developed symptoms of basal stem rot and brown root rot with retarded growth of the main roots. During the rainy season, numerous leaves of infected plants showed symptoms of brown spots which turned yellowing and fell to the ground prematurely.

**Characteristics of pathogen.** A total of 10 isolates of *P. nicotianae* were isolated from the diseased leaves, basal stems and roots of rehmannia plants grown in 3 fields (Table 1). All the 10 isolates belonged to $A^1$ mating type and Ko's sexuality type 1 [(12)], which were capable of forming oospores induced by the $A^2$ tester of *P. nicotianae* p731. Meanwhile they can stimulate the $A^2$ tester to form their own oospores. The colonies of these isolates showed slightly mosaic patterns on 5% CV8A in Petri dishes (Fig. 2C). Sporangiophores were branched simple sympodially. Sporangia were rare on V8A in Petri dishes but were abundant in water (Fig. 3G). Sporangia were mostly spherical to ovoid, unsymmetrical, non-deciduous and with one semi-spherical papilla. The sporangial sizes of the tested isolate *P. nicotianae* p28065 were listed in Table 2. Chlamydospores (Fig. 3H) were abundant. Oogonia were with smooth wall, and each oogonium was attached by one amphigynous antheridium (Fig. 3I). Oospores were spherical. The diameters of oogonia, oospores and antheridia of this isolate were 22.5-(27.0)-32.5 μm, 18.8-(23.3)-30 μm and 7-(9.5)-12.5 μm × 10.5-(13.1)-15 μm, respectively. The minimum, optimum and maximum temperatures for mycelial growth were 8, 32 and 36℃, respectively (Fig. 4). All the 10 isolates obtained from rehmannia plants belong to typical type of *P. nicotianae* according to Tucker's taxonomy [(17)] and Waterhouse's key [(19)].

**Pathogenicity.** Young plants of rehmannia (about 10 cm high, 1-3-month-old) were highly susceptible to infection by the isolates of *P. nicotianae*. The inoculated plants were killed after spraying with zoospore suspension for 5-10 days, whereas the controls sprayed with distilled water remained healthy. *Phytophthora nicotianae* was reisolated from all the inoculated plants.

## DNA sequences of ITS1/5.8S/ITS2 and partial β-tubulin regions

The information of nucleotide sequences of the ITS (ITS1-5.8SrDNA-ITS2) region and the partial sequence of the β-tubulin gene of representative *Phytophthora* isolates obtained from the medicinal herbs in Taiwan were listed in Table 3. The lengths of ITS sequences of the isolates p24148 (*P. cryptogea*), p26082 (*P. drechsleri*) and p28065 (*P. nicotianae*) were 798 bp, 798 bp and 803 bp, respectively. The lengths of the partial sequence of the β-tubulin gene for all the three isolates were 658 bp. All the query sequences were submitted to NCBI BLAST sever and the accession numbers of their respective best hit (including names of the organisms) were listed in Table 3. The identities between each query sequence and its best hit are within 99.5-100% for the ITS regions and 99.5-100% for the partial β-tubulin gene. All the sequences of representative isolates have been submitted to the GeneBank and the accession numbers were listed in Table 3.

Table 3. The GeneBank accession number and BLAST searching results of nucleotide sequence of ITS1-5.8S rDNA-ITS2 region and partial β-tubulin gene[1] of *Phytophthora* isolates from Chinese medicinal herbs in Taiwan

| *Phytophthora* species | Isolate number | Host | Gene | Length (bp) | GenBank accession no. | BLAST searching [2] | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | GenBank best hits | | Identity (%) (identical bp no. /total bp no.) |
| | | | | | | Accession no. | Microorganism species | |
| *P. cryptogea* | p24148 | *Salvia miltiorrhiza* | ITS | 798 | GU111624 | GU993890.1 etc 6 sequences | *P. cryptogea* | 99.5-100 (794-798/798) |
| *P. drechlsler* | p26082 | *Astragalus membranaceus* | ITS | 798 | GU111629 | GU259056.1 etc 4 sequences | *P. drechsleri* | 99.87-100 (797/798) |
| *P. nicotianae* | p28065 | *Rehmannia glutinosa* | ITS | 803 | JX465722 | JF792541.1 etc 33 sequences | *P. nicotianae* | 100 (803/803) |
| *P. cryptogea* | p24148 | *S. miltiorrhiza* | β-tubulin | 658 | JX629272 | HQ455650.1 etc 3 sequences | *P. cryptogea* | 99.54-99.70 (655-656/658) |
| *P. drechlsleri* | p26082 | *A. membranaceus* | β-tubulin | 658 | JX629273 | HQ455662.1 etc 2 sequences | *P. drechsleri* | 100 (658/658) |
| *P. nicotianae* | p28065 | *R. glutinosa* | β-tubulin | 658 | JX629274 | GU191318.1 | *P. nicotianae* | 99.85-100 (657~658/658) |

[1] The partial sequence of β-tubulin gene of *Phytophthora* was analyzed by the forward primer BT5 and the reversed primer BT6 developed by Villa *et al*. (17)

[2] The sequences were uploaded directly to NCBI (http://www.ncbi.nlm.nih.gov) and compared with sequences in GenBank databases using BLAST software.

## DISCUSSION

Many species of *Phytophthora* are important plant pathogens causing serious diseases on many crops in the world[8]. In Taiwan, Phytophthora diseases are very common and serious due to warm and humid climate which is favorable for the dissemination of the pathogens and the development of the diseases [4,9]. Dan-shen, astragalus, and rehmannia are widely used in Taiwan as medicinal herbs for the improvement of human health but there are no formal reports on Phytophthora diseases on these crops in Taiwan. In this study, three new Phytophthora diseases were identified on Chinese medicinal herbs, including *P. cryptogea* on dan-shen, *P. drechsleri* on astragalus, and *P. nicotianae* on rehmannia. None of these *Phytophthora* spp. have been reported to infest these Chinese medicinal herbs in any other country previously, except for *P. drechsleri* which was reported on *Astragalus membranaceus* in Korea [14], according to the records in the book "Phytophthora Diseases Worldwide" [8] and the database of the Systematic Mycology and Microbiology Laboratory, Agricultural Research Service, (SMML) USDA (http://nt.ars-grin.gov/ fungaldatabases/ index.cfm).

According to previous reports, *P. nicotianae* was the most common and destructive species in the genus of *Phytophthora* in the world [8] as well as in Taiwan [9]. The host range of this species of *Phytophthora* was very wide. According to the records in SMML, *P. nicotianae* attacked 890 species of higher plants in 255 genera in the world and 113 species of higher plants in 93 genera in Taiwan (unpublished data). In particular, most tested isolates of *P. nicotianae* obtained from different host plants could cause severe diseases on other hosts in cross-inoculation studies [1, 2, 3, and unpublished data]. Meanwhile, Taiwan is located in the tropical and sub-tropical region and the weather conditions are suitable for disease development of *P. nicotianae*, which has maximum, optimum and minimum temperatures of 36-37, 24-28-32, and 10℃, respectively. According to the traditional classification, *P. nicotianae* is categorized into Waterhouse's group II [19]. All the isolates of this species obtained from rehmannia in this study belong to the typical types, as their major morphological characteristics fit the descriptions in Tucker 's taxonomy [17], Waterhouse's key [19] and Stamp's tabular key [15].

Both *P. cryptogea* and *P. drechsleri* are categorized into Waterhouse's group VI [19], indicating that the sporangia formed by these two species are non-papillate. Therefore, these two species can be distinguished easily from *P. nicotianae*, because the later produced semi-spherical papillated sporangia. However, it is very difficult to distinguish between *P. cryptogea* and *P. drechsleri* based on the limited morphological characteristics [8]. In this study, the sporangia obtained from isolates of these two species are similar in morphology (ovate to obpyriform, non-papillate and non-deciduous), size, and arrangement on sporanigophores (simple sympodial) (Fig. 3, Table 2). Additionally, the Taiwanese isolates of *P. cryptogea* and *P. drechsleri* did not form sexual organs, although the original description given in the Waterhouse's key indicated that both species of the type specimens could produce oospores by single cultures without mating. The only way to distinguish the two species by traditional characteristics is based on their respective character of growth response to temperatures. The maximum temperature for mycelial growth is 32-33℃ for *P. cryptogea*, but 36-37℃ for *P. drechsleri* [15]. Therefore, the astragalus isolates were identified as *P. drechsleri* because they can grow at 36 ℃, while the dan-shen isolates were classified as *P. cryptogea*, because they can grow at or below 32-33℃ (Fig. 3).

It is difficult and disadvantageous to identify some *Phytophthora* species based only on morphological and physiological characteristics, because the available and critical morphological characteristics categorized for identification are very rare, the morphological features are variable and overlapping under different environmental conditions and the identification skills are variable. The evidence of molecule data are objective with fewer variables and, therefore, the molecule techniques are frequently used in recent years as supportive tools for identification of fungal species including *Phytophthora* spp. ITS1 and ITS2 of the ribosomal DNA are

frequently used for the identification of *Phytophthora* species [6, 7]. The sequences of ITS regions are well conserved at the intra-species (isolates or strains) level of *Phytophthora* and *Pythium*, but there is relatively high variability at the inter-species level for most *Phytophthora* species [6, 7]. Another conserved gene is β-tubulin, of which a partial sequence of 658 bp amplified with BT5 and BT6 primers was useful for the study of phylogenetic relationships among/within *Pythium* and *Phytophthora* [18]. Currently, the molecular data of *Phytophthora* species collected in the GenBank in NCBI website are relatively rich. It can assist in the identification of the pathogens. In this study, the molecular data of the ITS region and the β-tubulin gene of *P. nicotianae, P. cryptogea* and *P. drechsleri* from the medicinal herbs in Taiwan are consistent with the results obtained from traditional classification, which was based on morphological and physiological characteristics of these isolates. While analyzed by BLAST, the ITS1-5.8SrDNA-ITS2 sequences of *Phytophthora* isolates obtained in this study were found to be identical or near identical (with minute difference owing to dimorphic of the surveyed isolates) to the sequences of their respective best hit (Table 3), while the β-tubulin sequences showed an identity ranging 99.5-100% (Table 3). These results suggest that molecular data such as ITS and β-tubulin gene are useful for effective identification of *Phytophthora* species.

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## LITERATURE CITED

1. Ann, P. J. 1992. Phytophthora diseases of ornamental plants in Araceae in Taiwan. Plant Pathol. Bull. 1:79-89.
2. Ann, P. J. 2008. Diagnosis and identification of Taiwan *Phytophthora*. P17-32. In the Proceeding of Symposium on the Diagnosis and Identification of Major Pathogens in Plant Protection and Quarantine. BAPHIQ and National Chung Hsin University Pub. Taichung. 2008,9,9. (in Chinese with English abstract)
3. Ann, P. J., Kunimoto, R., and Ko, W. H. 1990. Phytophthora wilt of carnation in Taiwan and Hawaii. Plant Prot. Bull. 32:145-157
4. Ann, P. J., Wong, I. T., and Tsai, J. N. 2008. Phytophthora diseases of medicinal herbs in Taiwan. Plant Pathol. Bull. 17:.78-79. (abstract in Chinese)
5. Ann, P. J., Wong, I. T., and Tsai, J. N. 2010. New records of Phytophthora diseases of aromatic crops in Taiwan. Plant Pathol. Bull. 19:53-68
6. Cooke, D. E. L., and Duncan, J. M. 1997. Phylogenetic analysis of *Phytophthora* species based on ITS1 and ITS2 sequences of the ribosomal RNA repeat. Mycol Res 101:667–677.
7. Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G., and Brasier, C. M. 2000. A molecular phylogeny of *Phytophthora* and related Oomycetes. Fungal Gen. Biol. 30: 17-32.
8. Erwin, D. C., and Ribeiro, O. K. 1996. Phytophthora Diseases Worldwide. APS press, St. Paul. Minnesota, 562 pp.
9. Ho, H. H., Ann, P. J., and Chang, H. S. 1995. The Genus *Phytophthora* in Taiwan. Acad. Sin. Mon. Ser. 15. Taipei, Taiwan, ROC. 86 pp.
10. Hwang, S. C., Ko, W. H., and Aragaki, M. 1976. A simplified method for sporangial production by *Phytophthora cinnamomi*. Mycologia 68:1233-1234.
11. Ko, W. H. 1978. Heterothallic *Phytophthora*: evidence for hormonal regulation of sexual reproduction. J. Gen. Microbiol. 107:15-18.

12. Ko, W. H. 1980. Hormonal regulation of sexual reproduction in *Phytophthora*. J. Gen. Microbiol. 116:459-461.
13. Ko, W. H., Chang, H. S., and Su, H. J. 1976. Isolates of *Phytophthora cinnamomi* from Taiwan as evidence for an Asian origin of the species. Trans. Br. Mycol. Soc. 72:353-358.
14. Ryu, K.Y., Kim, J. S., and Hahm, Y. I. Downy Mildew of *Astragalus membranaceous* Burge Caused by *Peronospora trifoliorum* de Bary. Plant Pathol. J. 17: 308-310.
15. Stamp, D. J., Waterhouse, G. M., Newhook, F. J., and Hall, G. S. 1990. Revised Tabular Key to the Species of *Phytophthora*. Mycol. Pap. 162, Comm. Mycol. Ins. Kew Surrey, England.
16. Tsu, S. T. *et. al.* (eds.) 2002. List of Plant Diseases in Taiwan (4th Ed). Taiwan Phytopathol. Soc. Pub. Taichung. 386pp.
17. Tucker, C. M. 1931. Taxonomy of the genus *Phytophthora* de Bary. Mo. Agric. Exp. Sta. Res. Bull. 153. 208 p.
18. Villa, N. O., Kageyama, K., Asano, T., and Suga, H. 2006. Phylogenetic relationships of *Pythium* and *Phytophthora* species based on ITS rDNA, cytochrome oxidase II and β-tubulin gene sequences. Mycologia 98:410–422.
19. Waterhouse, G. M. 1963. Key to the species of *Phytophthora* de Bary. Mycol. Pap. 92, CMI, Kew Surrey, England.
20. Waterhouse, G. M. 1970. The genus *Phytophthora* de Bary- diagnosis (or descriptions) and figures from the original papers. Mycol. Pap. 122. CMI, Kew Surrey, England.
21. White, T. J., Bruns. T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of ribosomal RNA genes for applications. P. 315-322 *in* PCR Protocols: A Guide to Methods and Applications. Innis, M.A., Gelfand, D. H., Snirsky, J. J., and White, T. J. (*eds*) Academic Press, San Diego, USA.

# 摘 要

安寶貞[1,2]、王姻婷[1]、蔡志濃[1]、黃鴻章[1]. 2012. 台灣藥用植物疫病之新紀錄. 植病會刊 21:65-77.
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自 2001-2009 年調查台灣的藥用植物疫病，發現有三種未曾正式報告之疫病新紀錄，包括 *Phytophthora cryptogea* 爲害丹參 (*Salvia miltiorrhiza*)，*P. drechsleri* 爲害黃耆 (*Astragalus membranaceus*)，以及 *P. nicotianae* (= *P. parasitica* ) 爲害地黃 (*Rehmannia membranaceus*)。分述如下：*P. cryptogea* 爲害丹參的莖基部與根系，造成組織褐變腐敗，葉片下垂萎凋，罹病植株最終死亡；*P. drechsleri* 感染黃耆小葉、莖部及根系，造成莖、葉腐敗與植株萎凋死亡。地黃對 *P. nicotianae* 非常感病，全株均可被感染，罹病植株在短期內大量死亡，這些病害都在夏秋季降雨季節發生。所有從丹參、黃耆及地黃分離到的疫病菌株均爲 $A^1$ 配對型 (mating types)。在溫室，將各寄主植物分離到疫病菌的游走子懸浮液接種到寄主植物，均會產生與田間相同的病徵，而且相同的疫病菌均可自接種後發病的寄主組織上分離得到，證明這些保健植物罹患疫病分別由上述三種疫病菌引起。此外，分析上述三種疫病菌的核醣體內轉錄區間 (ITS region, ITS1-5.8SrDNA-ITS2) 與 β 微管蛋白基因 (β-tubulin) 的部份 DNA 序列，亦支持這三種疫病菌的分類地位。這三種疫病菌中，除 *P. drechsleri* 爲害黃耆在韓國有記錄外，其餘 *P. cryptogea* 爲害丹參與 *P. nicotianae* 爲害地黃在世界其他各地尚未發表過。

關鍵詞：疫病新紀錄、藥用植物、丹參、黃耆、地黃、*Phytophthora cryptogea*、*P. drechsleri*、*P. nicotianae*、台灣